1054-138 Myeloperoxidase Containing Macrophages and Neutrophils and Stainable Intracellular Iron in Plaque Progression to Rupture and Organize

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Background. MPO-containing macrophages have been described at sites of plaque rupture. The specificity of this marker for plaque rupture is unknown, and the presence of neutrophils or iron-containing macrophages at plaque rupture sites has not been described.
Methods. We studied 16 acute ruptures, 10 organizing ruptures, 29 thin cap fibroatheromas, and 14 fibroatheromas from sudden coronary death victims by immunohistochemical and histochemical techniques. Inflammatory cells were typed with anti-CD68 (macrophages), anti-BP-30 (neutrophil bactericidal glycoprotein), and anti-MPO. Iron was localized by Mallory's Prussian blue stain.
Results. MPO positive cells were present in the majority of ruptured caps, but only few non-ruptured caps (table). Iron containing foam cells were present in the caps of 93% of acute ruptures, of 84% of organizing ruptures, 32% of thin cap atheromas, and 10% of fibroatheromas. In addition, there were deeper areas of hemosiderin deposition in 50% of acute and organizing ruptures, 14% of fissures, 36% of thin cap atheromas, and 27% of fibrous cap atheromas.
Conclusion. Although MPO positive macrophages and neutrophils are present in the majority of acute ruptures, neutrophils appear to be a transitory infiltrate. Iron-containing macrophages are present at acute ruptures as well as healed ruptures with organization and may represent a means of detecting sites of acute events in the coronary segments.

Cap type	% with neutrophils	Neuts./ mm2	MPO+ cells/mm2	% MPO + cells = neuts.	Iron macrophages/ mm2
Fibrous	0	0	0	0	1.2
Thin cap	12	2 ± 2	13 ± 5	22 ± 14	20 ± 13
Rupture	89	90 ± 32	127 ± 29	65 ± 23	88 ± 44
Organized rupture	11	5 ± 5	47 ± 20	11 ± 11	90 ± 41

1054-139 Importance of Methodology in Chlamydia Pneumoniae Serology

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Background: An association between Chlamydia pneumoniae seropositive status and coronary heart disease remains controversial. We examined the concordance between 4 commonly used enzyme immuno asssays and the microimmunofluorescence test, the gold standard method, and investigated whether the choice of assay influenced antibody seroprevalence in patients with coronary atherosclerosis and healthy individuals.
Methods: MRL Chlamydia MIF IgG test (MIF), Labsystems Chlamydia pneumoniae IgG EIA (LS), R-Biopharm Elegance Chlamydia pneumoniae IgG EIA (RB), Medac Chlamydia pneumoniae IgG sELISA (MCp) and Medac Chlamydia IgG rELISA (MC) were tested on sera from 112 healthy men. Male patients with angiographically defined coronary artery disease were also investigated using the LS and MCp test.
Results: The agreement between LS (73/112, 65 %) or MC (55/112, 49 %) and MIF (89/112, 79 %) was moderate to fair (kappa = 0.583; kappa = 0.235). MCp (kappa = 0.679) and RB (kappa = 0.665) showed good agreement with MIF, with 90/112 (80 %) and 87/112 (78 %) of controls reacting positive. Furthermore, a significant difference in seroprevalence between patients and healthy subjects was observed with the LS assay, but not with the MCp test .
Conclusion: The concordance between MIF and other commonly used serologic assays for Chlamydia pneumoniae antibody detection is good to fair. The choice of serological assay is important when evaluating whether Chlamydia pneumoniae seropositivity is related to coronary artery disease.

test	Seropositive controls (%)	Seropositive patients (%)	Seropositive patients (%)	p value
LS	71/112 (63.4)	68/82 (82.9)	patients with diffuse atherosclerosis, 1, 2 or 3 vessel disease	0.003
MCp	90/112 (80.4)	87/98 (88.8)	patients with diffuse atherosclerosis, 1, 2 or 3 vessel disease	0.094
LS	71/112 (63.4)	61/73 (83.6)	patients with 1,2 or 3 vessel disease	0.003
MCp	90/112 (80.4)	79/89 (88.8)	patients with 1,2 or 3 vessel disease	0.106

1054-140 Reactive Oxygen Species Contribute to Inflammation-Induced Endothelial Dysfunction

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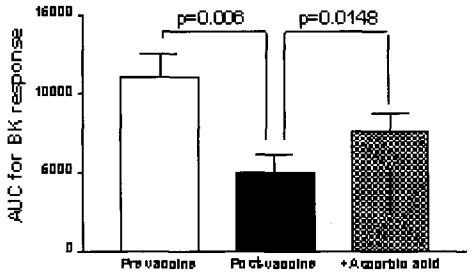
Background: Infection/inflammation might increase the risk of vascular events by inducing endothelial dysfunction. We tested the hypothesis that acute inflammation impairs the release or action of atheroprotective nitric oxide (NO) from the endothelium by limiting the availability of substrate or enhancing the generation of reactive oxygen species.
Methods: Forearm blood flow was measured using venous plethysmography in 18 healthy volunteers (age 28±6 years). Dose-response curves to intra-arterial L-NMMA (1-4 umol/min), noradrenaline (NA; 60-240 pmol/min), bradykinin (BK; 20-80 pmol/min) and glyceryl trinitrate (GTN; 8-32 nmol/min) were constructed before and 8 hours after administration of typhoid (Typhim Vi) vaccine to generate an inflammatory response. In others responses to BK and GTN were repeated after intra-arterial infusion of L-arginine (substrate for NO; 50umol/min n=5) or anti-oxidant vitamin C (25mg/min n=8).
Results: Vaccination induced a cytokine response and reduced dilatation to endothelium-dependent agonists BK (p=0.006) and L-NMMA (p<0.0001) without affecting the response to GTN (p=0.36) or NA (p=0.62). Post vaccine, blood flow responses to BK (but not GTN) were partially reversed by vitamin C (fig) but unaffected by L-arginine.
Conclusion: Inflammation reduces vascular NO bioavailability, an effect that is partially reversible with local anti-oxidants. These findings suggest a role for reactive oxygen species in inflammation-induced endothelial dysfunction.



1054-141 Differential Proliferative Index of Resident Smooth Muscle Cells and Infiltrating Leukocytes After Balloon Angioplasty and Stenting of Swine Coronary Arteries

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Background. Inflammation plays a central role in vascular lesion development after balloon angioplasty and stenting. Quantitative assessments of leukocyte infiltration and proliferation in injured vessels is lacking. To evaluate proliferation of resident smooth muscle cells (SMCs) and infiltrating leukocytes, we conducted a flow cytometry-based analysis of injured vessels. ***Methods.*** Coronary arteries of 5 juvenile swine were stented (RCA), balloon overstretched (LAD), or left intact (LCX). Naive arteries were used as controls. Animals were injected with 60 mg/kg BrdU 24 hours before sacrifice, which was performed 3 days after the intervention. After enzymatic dissociation, cell suspensions were immunofluorescently-labeled for CD45, BrdU and α-SM actin. Selected samples were stained for DNA content using 7-AAD. Stained samples were analyzed on a FACSCalibur flow cytometer. ***Results.*** The number of cells in stented and ballooned vessels was not significantly different from control arteries (naive: $1.56\pm0.34\times10^4$, LCX: $1.98\pm0.34\times10^4$, LAD: $1.75\pm0.25\times10^4$, RCA: $1.62\pm0.29\times10^4$ cells/mg tissue, means±SEM). A remarkable inflammatory infiltrate was detected 3 days after injury in ballooned and stented vessels (naive: 5.8±1.9%; LCX: 7.9±1.2%; LAD: 24.0±3.5%; RCA: 33.1±3.3%, p<0.001). The proliferative index of CD45+ cells in uninjured and injured vessels was similar but significantly higher than that in peripheral blood leukocytes (PBL) (naive: 15.9±1.5%; LCX: 15.6±4.9%; LAD: 15.3±1.5%, RCA: 14.5±1.3%, PBL: 3.9±0.3%, p<0.05). Unlike CD45+ cells, the proportion of proliferating SMCs in ballooned and stented vessels was significantly higher when compared to uninjured and naive arteries (naive: 3.63±1.0; LCX: 4.3±1.6; LAD: 33.2±4.5; RCA: 45.0±3.7, p<0.05) ***Conclusions.*** Our results document with precision the inflammatory cell involvement in the early response to balloon and stent injury. The differential rates of proliferation detected by flow cytometry demonstrate its sensitivity, and suggest that activated leukocytes may mediate the proliferative response of resident SMCs.

1054-142 Prostaglandin E1 Induces Vascular Endothelial Growth Factor-A in Human Adult Cardiac Myocytes and Coronary Artery Smooth Muscle Cells

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Background: Vascular Endothelial Growth Factor-A (VEGF-A) induces proliferation, migration and NO-synthesis in endothelial cells and is able to stimulate neoangiogenesis in ischaemic organs. A significant increase of VEGF-A serum levels was shown after myocardial infarction. These data suggest the importance of the VEGF system during repair and neovascularization. Recently we showed that Prostaglandin E1 (PGE1) induced angiogenesis in hearts of patients with ischaemic heart disease (Mehrabi et al. Cardiovasc. Res. 2002). In this study we aimed to investigate whether PGE1 affects the expression of VEGF-A in cultured human adult cardiac myocytes (HACM) or human cor-

onary artery smooth muscle cells (HCASMC), respectively. **Methods**: HACM were isolated from recipients' hearts after heart transplantation and characterized by positive staining for troponin-I and cardiotin. The cells were negative for two fibroblast-specific antibodies as well as for desmin and vWF indicating the absence of fibroblasts, smooth muscle cells and endothelial cells. HCASMC were isolated from recipients' coronary arteries using the explant-technique and stained positive for alpha-smooth muscle actin. Such characterized cells were treated with PGE1 and effects on VEGF-A expression were studied using an ELISA and RT-PCR. **Results**: When HACM or HCASMC were treated with PGE1, a significant up to threefold increase in VEGF-A production could be observed. These results could be confirmed on the level of specific mRNA expression as determined by RT-PCR. The effect of PGE1 on VEGF-A expression could be reversed by pertussis toxin. **Conclusion**: We conclude from our data that PGE1-induced expression of VEGF-A in cardiac myocytes and smooth muscle cells might contribute to its beneficial effects seen in patients with ischaemic heart disease. It should, however, be mentioned that increased production of VEGF in smooth muscle cells could potentially also induce angiogenesis within the atherosclerotic plaque and thereby could contribute to plaque destabilization.

1054-143 **Different Cell Death Rates Determine Growth of Vascular Smooth Muscle Cells From Human Aortocoronary Bypass Vessels: Implications for Patency of Radial Artery Grafts**

Karin Frischknecht, Helen Greutert, Thierry P. Carrel, Felix C. Tanner, University Hospital Bern, Bern, Switzerland

Long-term patency of aortocoronary bypass grafts is determined by vascular smooth muscle cell (VSMC) proliferation leading to neointima formation. Radial artery (RA) patency rates seem to be in between those from mammary artery (MA) and saphenous vein (SV). We therefore examined whether and, if so, why proliferation of VSMC from these bypass vessels differs accordingly. After 6 days of serum stimulation, RA-VSMC number (51'520±7'538) was lower than SV (69'750±11'588), but higher than MA (28'365±6'324) (SV vs. MA: $p<0.01$; SV or MA vs. RA: p=n.s.; n=4). In contrast, RA-VSMC exhibited only minimal proliferation to PDGF-BB, (15'493±2'116), which was comparable to MA (15'446±2'768; MA vs. RA: p=n.s.) and differed from SV (33'094±3'028; SV vs. RA or MA: $p<0.01$; n=4). Analogous results were obtained by 3H-thymidine incorporation (n=4). As determined by FACS analysis, PDGF receptor β expression was similar (p=n.s.; n=5), whereas PDGF receptor β expression was higher in RA as compared to MA or SV (RA vs. MA or SV: $p<0.05$; MA vs. SV: p=n.s.; n=5). Western blotting confirmed these findings. Propidium iodide incorporation after PDGF stimulation showed identical cell cycle distribution in VSMC from all three vessels. Similarly, Western blotting for cell cycle proteins after PDGF treatment revealed identical expression: the cyclin-dependent kinase inhibitor (CKI) p27 was downregulated, the CKI p21 was slightly induced, while the CKI p57, cyclin-dependent kinase 2 (cdk2) and cyclin E did not show any change. Cdk2 kinase assay confirmed that G1 progression was identical in VSMC from all three vessels. While neither the visual aspect nor trypane blue staining were different, LDH release was higher in VSMC from MA as compared to RA or SV (SV vs. MA: $p<0.05$; RA vs. MA or SV: p=n.s., n=5). Thus, cell cycle progression is identical in VSMC from human bypass vessels, while different cell death rates determine growth, suggesting that VSMC death rather than proliferation may account for the different patency rates of bypass grafts. The weak effect of PDGF in RA as compared to SV encourages the clinical use of RA grafts.

1054-144 **Activated Protein C Inhibits the Release of Proinflammatory Cytokines and Chemokines From the Monocytic Cell Line THP-1**

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Background: It has been suggested that the activated protein C (APC) pathway serves as a common link between coagulation and inflammation. This has been further supported by reports showing that APC could inhibit or induce cytokine production in endothelial cells. In addition to endothelial cells, monocytes are also important in the inflammatory response. In order to determine what effect APC may have on cytokine and chemokine production in monocytes, experiments were conducted using the monocytic cell line THP-1. We investigated the effect of human APC on the monocytic release of the proinflammatory cytokines macrophage inflammatory protein-1-alpha (MIP 1-alpha) and tumor necrosis factor-alpha (TNF-alpha) as well as the effect of APC on the release of the chemokine monocyte chemoattractant protein-1 (MCP-1). All of these mediators may be important for the interaction of monoytes at the endothelium during initiation and progression of atherosclerosis. **Methods and Results**: We established a monocytic cell model of inflammation by the addition of lipopolysaccharide (LPS from E.coli 026:B6; 0.1-1μg/ml) and examined the effect of APC on cytokine/chemokine release. As a further extension of the effect of APC on cytokines, we found that human APC (2.5-10μg/ml) inhibited LPS-induced release of MIP-1-alpha and MCP-1 from THP-1 cells, as measured by an Enzyme-linked immunosorbent assay at 6 up to 24 h. Furthermore APC inhibited the LPS-induced relase of TNF-alpha, time- and dose-dependently.**Conclusions:** The ability of APC to inhibit the release of the proinflammatory cytokines MIP-1-alpha and TNF-alpha and of the chemokine MCP-1 provides further evidence that APC may modulate initiation and progression of atherosclerotic lesions through the control of cytokine and chemokine release from monocytes.

1054-145 **The Coexpression of the Migration Inhibiting Factor 1-Alpha With CD40l in Human Atherosclerotic Plaques Is Associated With Advanced Plaque Stages and Extent of Intraplaque Inflammation**

Alexander Schmeisser, Thomas Illmer, Christiane Graffy, Herribert Loesel-Sadee, Dittmar Boeckler, Dittmar Menschikowski, Gerhard Ehninger, Christoph Garlichs, Werner G. Daniel, Ruth H. Strasser, Technical University of Dresden, Dresden, Germany, University Erlangen-Nuernberg, Erlangen, Germany

Background: MIF is a hormone and cytokine with pivotal importance in inflammatory and immune responses. Little is known about the role of MIF in atherosclerotic plaque progression and inflammation.
Methods: Carotid endartherectomy and vessel samples (n=46) were collected from 36 patients and 4 young accident victim controls and histological classified by Stary: minimal plaques (MP) were defined as Stary: 0-III and advanced plaques (AP) as Stary: IV-VIII. The immunhistochemical detection of MIF and CD40l in macrophages (Ma), microvascular endothelial cells (MEC), vascular smooth muscle cells (VSMC) and T-lymphocytes (TL) was perfomed. The relative extent of MIF expression was graded on scales of d0 to d3 in terms of specific staining intensity. In addition, the number intraplaque microvessels and inflammatory foci (>10 CD45-positive mononuclear cells (MNC)/field) were counted. In vitro, the monocyte cell line THP-1 was stimulated with a CD40l-fusion protein (1microg/ml), 1mg/microl LPS or with heat denaturated CD40l.
Results: APs (n=36) shows sign. ($p<0.01$) more intimal MNC and intraplaque microvessels than MPs from patients/controls (n=10), and higher MIF expressing intensitiy by MA/TL and MVEC. Strong MIF staining (d2/d3) was detected in Ma/TL in 70% and MEC in 80% of AP, and only in 10% and 0% of MP, respectively. Strong MIF staining was colocalized with CD40l expression in foci with Ma/TL staining. VSMC stained only weak or no for MIF. CD40l upregulated THP-1 MIF expression after 12h (mRNA) and 48h (protein) sign. ($p<0.01$) and comparable to TNF-alpha in vitro.
Conclusion: The results reveal a marked upregulation of migration inhibitory factor 1-a in close correlation to macrophage/T-cell infiltration and intraplaque microvessel extent in advanced plaques. Additionally, the coexpression with CD40l suggests a pivotal role of MIF in the inflammatory process and intimal neoangiogenesis of atherosclerotic plaques.

POSTER SESSION

1055 Basic Mechanisms of Angiogenesis and Arteriogenesis

Sunday, March 30, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1055-117 **Hammerhead Ribozymes Directed Against Cyclin E and E2F1 as Tools to Prevent In-Stent Restenosis**

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In-stent-restenosis is associated with prolonged cellular proliferation in stented arteries. We explored the possibility to specifically inhibit vascular smooth muscle cell (VSMC) proliferation via hammerhead ribozymes. Two ribozymes, targeted against cyclin E and E2F1 mRNAs, were used. As these two genes co-operate to promote the transition from G1 to S phase of the cell cycle, they represent ideal targets to arrest cells in G1 phase thus blocking proliferation.
Methods: Phosphorothioate-modified ribozymes complexed with liposomes were administered to human VSMC. Cell proliferation and distribution throughout the phases of the cell cycle were analyzed by a double DNA staining procedure. The antiproliferative effect of the specific ribozymes were evaluated in comparison to scrambled binding arm ribozymes (RzScr) liposomes alone and non-treated cells. Different ribozyme concentrations and times after transfection were tested. In addition, the effect of a combined administration of the two ribozymes was evaluated. Finally, a comparison between the efficacy of ribozymes and the corresponding antisense oligonucleotides (ODNs) was performed.
Results: Two days after transfection, each of the two specific ribozymes showed a dose-dependent inhibition of proliferation in comparison to non-treated, liposome-treated and RzScr treated cells. Ribozyme effect peaked at 420 nM, reducing cell growth down to 34 % of non treated cells. At the same final concentration (420 nM), a combined administration of the two ribozymes (210 nM each) resulted in an even more pronounced proliferation inhibition (25 % of non treated cells). Time course experiments showed that ribozyme activity persists for at least four days following transfection. In addition, ribozymes were more effective than the respective antisense ODNs. Finally, we proved that growth inhibition was due to an increase in G1 phase cells, paralleled by the concomitant decrease in S phase cells.
Conclusion: Ribozymes against cyclin E and E2F1 reduced VSMC proliferation up to four days by arresting cells in G1 phase. The dramatic and specific decrease in cell growth provides the basis for the development of a ribozyme therapy for in-stent restenosis.